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Reviewer: S. Semalulu , **Date** April 10, 2001

STUDY TYPE: Sub-chronic oral Toxicity - mice [feeding]; OPPTS 870.3100 [§82-1]; OECD 408.

TEST MATERIAL (PURITY): SZX 0722 (99.4%) [Iprovalicarb]

SYNONYMS: Melody

CITATION: B. Watta-Gebert and A. Popp (1996): SZX 0722 - Dose-Range-Finding Study in B6C3F$_1$ mice (administration in food over 13 weeks). Bayer AG, Report no. 25296. Unpublished.

SPONSOR: Bayer Corporation.

EXECUTIVE SUMMARY:

In a dose-range finding sub-chronic toxicity study (MRID not available), SZX 0722 technical (98.1 - 98.7 %) was administered to B6C3F1 mice (6 or 10/sex/group) in the diet at dose levels of 0, 280, 1400, 7000, 1400 ppm (0, 63.9, 325.0, 1724.6, and 3473.1 or 0, 125.2, 696.5, 3599.5, and 6869.0 mg/kg bw/day for males and females, respectively) for 13 weeks.

There were no treatment related clinical signs, nor effects on body weight development, or food intake. Water intake per animal and water intake relative to the body weight gain were elevated in males at 7000 ppm and above, and in females at 14000 ppm.

Haematological investigations revealed a slight increases in the mean corpuscular volume in males beginning at 7000 ppm, and slight decreases in the erythrocyte count (in both sexes), haematocrit (females only), and as well as the mean corpuscular haemoglobin (both sexes), and mean corpuscular haemoglobin concentration (females only) at 14000 ppm. Plasma cholesterol levels were increased in females at 7000 ppm and above. Slight increases in absolute liver weights were observed in both sexes at 14000 ppm. Relative liver weights were increased in females beginning at 7000 ppm, and in males at 14000 ppm. The changes in liver weights of females were accompanied by alterations in plasma cholesterol and was assessed as indicative of early liver effects. There were decreases in absolute and relative kidney weights in males at 14000 ppm, but there was no accompanying histopathology in the kidneys and therefore considered incidental. Histopathological investigations did not provide any evidence of changes in any tissue related to treatment with SZX 0722.

The LOAEL in males was 7000 ppm, based on elevated water intake and changes in haematological parameters (erythrocyte count, MCV). The NOAEL in males was 1400 ppm (325.0 mg/kg bw/day). The LOAEL in females was 7000 ppm, based on increases in liver weights, and plasma cholesterol levels. The NOAEL in females was 1400 ppm (696.5 mg/kg bw/day).

Dose levels for the long term study selected based on the findings from this study were appropriate.

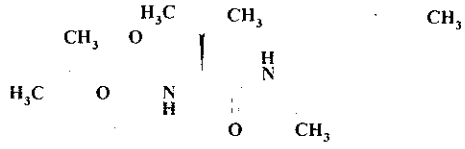
This short term toxicity study is classifiable as acceptable supplementary study because it was a dose-range finding study for a long-term toxicity study in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. Only 6 animals/sex were used in the fourth and highest treated group, instead of the usual 10 animals/sex/dose. The study had three other treated groups with 10 animals/sex/dose, and its purpose was dose-range finding, for a long-term toxicity study. Therefore the deviations do not affect the acceptability of this study.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** SZX 0722

Description:	Technical, a white powder
Lot/Batch #:	898222005
Purity:	98.1 -98.7 % a.i.
Compound Stability:	Stable at room temperature
CAS #:	140923-25-7
Structure	

2. **Vehicle and/or positive control:** 1 % (DAB 10) Peanut oil

3 **Test animals:**

Species:	Mice	
Strain:	B6C3F$_1$(SPF)	
Age/weight at study initiation:	5 week old, 21-26 g males; 15 -19g females	
Source:	Bomholtgard Breeding and Research Centre Ltd. 8680 Ry, Denmark	
Housing:	Individually caged in Type II Macrolon cages, on low dust wood shavings.	
Diet:	Altromin fixed formula standard diet 1321 (Altromin, GmbH, Lage), fed *ad libitum*	
Water:	Tap water in 300 ml polycarbonate bottles, provided *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 2 °C
	Humidity:	55 ± 5 %
	Air changes:	15-20/hr
	Photoperiod:	12 hrs dark/ 12 hrs artificial light
Acclimation period:	1 week	

B. STUDY DESIGN:

1. **In life dates** - Start: April 1993: July 1993.

2. **Animal Assignment/Dose Levels:** Animals were randomly assigned to the test groups noted in Table 1 (using computer generated random numbers).

TABLE 1: STUDY DESIGN

Test Group	Dose. in Diet (ppm)	Dose to animal (mg/kg bw/d)		number of animals	
		male	female	Male	Female
Control	0	0	0	10	10
Low (LDT)	280	63.9	125.2	10	10
Mid (MDT)	1400	325	696.5	10	10
High 1(HDT1)	7000	1724.6	3599.5	10	10
High2 1(HDT2I)	14000	3473.1	6869	6	6

3. Dose Selection: The doses were selected based on findings from two preliminary studies using 3-4 animals/group, at dose levels of 7500, 14000 or 20000 ppm of SZX 0722 in the diet for 7 days, which indicated only a slight decrease in body weight even at 14000 ppm and above, well above the limit dose of 7000-10000 ppm which is recommended for the mouse oncogenicity study.

4. Diet preparation and analysis:

Diets were prepared weekly, by mixing appropriate amounts of test substance with Altromin 1321 Meal and were stored at ambient temperature. To all diet mixtures, peanut oil (10g/kg food) was added to minimize dust formation. Homogeneity and stability were tested on 5 food sample samples collected directly after each diet preparations. Samples of treated food for the three nominal concentrations were analysed for concentration and stability after storage for 14 days under conditions comparable to the study rooms.

Results -
Results of the analysis for homogeneity, stability and concentration, of the test material in food samples expressed as a percentage of the target concentration were as follows.

Homogeneity Analysis: 91 - 101% of nominal concentration.

Stability Analysis: 101 to 108% of nominal concentration. Stable for up to 14 days

Concentration Analysis: 92 - 105% of nominal concentration

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics -
Statistical evaluations for clinical chemistry, haematology, body, food and water consumption and organ weights were performed using SAS routines, by comparing treated groups with controls using either the Dunnet test, Kruskal Wallis test followed by Mann-Whitney-Wilcoxon (U Tests). Statistical significance between control and treated animals was set at $p \leq 0.05$ and $p \leq 0.01$.

C. METHODS:

1. Observations:

Animals were inspected twice daily (once on weekend and holidays) for signs of toxicity and mortality. A detailed physical examination was conducted once weekly. Food and water consumption were measured weekly. Blood samples from the retro-orbital sinus were collected from 10 animals/sex/dose at 13 weeks for clinical chemistry determinations.

2. Body weight

Animals were weighed before treatment and weekly thereafter.

3. Food consumption and compound intake:

Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/animal/day. Food efficiency was calculated as mean food intake per kilogram body weight per day and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination

No specific opthalmological examined was conducted beyond the general physical examination.

5. Haematology & Clinical Chemistry:

Blood samples were collected in weeks 12/13 from the retro-orbital sinus of non fasted animals under ether anaesthesia, from all dose groups for haematology and clinical analysis. The parameters checked (x) were examined.

a. Haematology

x	Hematocrit (HCT)	x	Leukocyte differential count
x	Hemoglobin (HGB)	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)	x	Mean corpusc. volume (MCV)
x	Platelet count	x	Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
x	Calcium	x	Albumin
x	Chloride	x	Creatinine
x	Magnesium	x	Urea nitrogen
x	Phosphorus	x	Cholesterol
x	Potassium		Globulins
x	Sodium		Glucose
		x	Bilirubin
	ENZYMES	x	Total serum protein (TP)
x	Alkaline phosphatase (ALK)	x	Triglycerides
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)		
x	Serum aspartate amino-transferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
x	Glutamate dehydrogenase		

Not required for carcinogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urine parameters were not examined.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the tissues checked (x) in the table below were collected for histological examination In addition, the organs (marked xx) in the table were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta	xx	Brain
x	Salivary glands	xx	Heart	x	Periph.nerve
x	Esophagus	x	Bone marrow	x	Spinal cord (3 levels)
x	Stomach	x	Lymph nodes	x	Pituitary
x	Duodenum	xx	Spleen	x	Eyes (optic n.)
x	Jejunum	x	Thymus		
x	Ileum				GLANDULAR
x	Cecum		UROGENITAL	xx	Adrenal gland
x	Colon	xx	Kidneys+	x	Lacrimal gland
x	Rectum	x	Urinary bladder	x	Mammary gland
xx	Liver+	xx	Testes+	x	Parathyroids++
x	Gall bladder	x	Epididymides	x	Thyroids++
x	Pancreas	x	Prostate		
		x	Seminal vesicle		OTHER
	RESPIRATORY	x	Ovaries+	x	Bone
x	Trachea	x	Uterus	x	Skeletal muscle
x	Lung			x	Skin
x	Nose			x	All gross lesions and masses
x	Pharynx				
x	Larynx				

II. RESULTS

A. Observations

1. Clinical signs of toxicity -

There were no treatment related clinical signs.

2. Mortality -

Survival rates of treated animals of both sexes in all dose groups were similar to the controls throughout the treatment period. There were no mortalities.

B. Body weight and body weight gain

Mean weekly body weights are presented in Table 2. Body weight in treated groups of both sexes were comparable to the controls throughout the study period. There were no treatment related effects on body weight development.

TABLE 2: Mean body weight (BW) and overall body weight gain (BWG) [g].

Week	0	280	1400	7000	14000
Males					
BW Wk 0	23	23.2	22.7	23.8	22.8
BW Wk 1	23.2	23.9	23.8	24.3	23.8
BW Wk 3	23.9	24.9	24.1	24.4	24.8
BW Wk 6	24.6	26.1	25.3	24.2	25.7
BW Wk 9	25.3	26.3	25.9	26.6	26.6
BW Wk 13	27.5	28.5	28	27.7	27.8
Overall BWG Wk -1-13	4.5	5.2	5.3	3.9	5
Females					
BW Wk 0	17.1	17.5	17.2	16.7	16.9
BW Wk 1	17.7	18.4	18.2	18.1	18.6
BW Wk 3	19.4	20	19.4	20.1	20.6
BW Wk 6	21	22	21.7	21.2	21.4
BW Wk 9	21.6	22.2	22.3	21.5	22.4
BW Wk 13	23.4	24.3	23.5	22.3	23.1
Overall BWG Wk -1-13	6.3	6.8	6.3	5.7	6.2

C. Food consumption and compound intake

1. Food and water consumption -

There were no treatment related differences in food consumption between treated groups and the controls. Males at 7000 ppm, and both males and females at 14000 ppm consumed more water per animal (12-16%) and more water per unit weight gained (10-12%) than controls. The changes in water consumption and water consumption per unit weight gained were considered treatment related, although its toxicological significance remained unclear.

2. Compound consumption (time-weighted average)

Compound intake is presented in Table 1. In all dose groups of both sexes, the test compound consumption was consistent with the nominal dose factor.

3. Food efficiency

There were no treatment related differences in food efficiency between treated groups and the controls.

Table 3. Water intake grams per animal and in grams per unit body weight gained

	0 ppm	280 ppm	1400 ppm	7000 ppm	14000 ppm
Males					
Water intake (g/animal)	5.1	5.4	5	5.7	5.9
Water intake (g/kg bw/day)	204.7	207.8	198.1	227.1	228.6
Females					
Water intake (g/animal)	5	5.2	5	5.2	5.7
Water intake (g/kg bw/day)	239.6	242.5	234	246.8	262.4

D. Ophthalmoscopic examination - There were no specific ophthalmological examinations conducted in this study.

E. Blood analyses

1. Haematology - Males at 7000 ppm had a slight but significant increase in mean corpuscular volume compared to controls. At 14000 ppm there was a slight decrease in erythrocyte count, and hematocrit in both sexes, and slight increases in mean corpuscular volume (males only). Females at 280, 7000 and 14000 ppm had slightly elevated thrombocyte counts compared to control. However, the mean thrombocyte values for the control females were below the historical control range, and the increase in thrombocyte count was not dose related, hence, not considered toxically significant.

Haematological findings

	Ery 10^{12}/l	HB g/l	HCT l/l	MCV fl	MCH pg	MCHC g/l	Thro 10^{9}/l	
Males								
0 ppm	9.7	150	0.448	46.2	15.5	335	1031	
280 ppm	9.62	150	0.443	46	15.6	340	977	
1400 ppm	9.58	150	0.447	46.6	15.6	335	983	
7000 ppm	9.52	149	0.448	47.1*	15.6	332	1005	
14000 ppm	9.31*	148	0.443	47.6*	15.9**	335	1030	
Females								
0 ppm	9.36	148	0.436	46.6	15.8	341	754	
280 ppm	9.34	148	0.435	46.5	15.8	340	832*	
1400 ppm	9.29	147	0.432	46.5	15.9	342	805	
7000 ppm	9.24	149	0.429	46.5	16.1	346	818*	
14000 ppm	9.03	146	0.42	46.6	16.2	349	851**	

* $P \leq 0.5$ % significance level ** $p \leq 0.01$

2. Clinical Chemistry -

Activities of plasma enzymes of treated groups were comparable to controls. Plasma cholesterol levels were increased in females at 7000ppm and above, and in males at 1400 and 7000 ppm, but not at 14000ppm. The increase in cholesterol levels of females , which was also accompanied by increased relative liver weights at the same doses, was considered treatement-related, but that in males was deemed incidental because of the lack of a dose relationship. Likewise blood urea levels of females were slightly elevated at 1400 and 7000 ppm, but not at 14000ppm, hence, considered incidental due to the absence of a dose relationship.

Table 4. Notable Clinical chemistry findings

Dose groups	0 ppm	350 ppm	1400 ppm	7000 ppm	14000
Males					
Chol [mmol/l]	3.2	3.23	3.55**	3.53*	3.13
Females					
Chol [mmol/l]	2.58	2.75	2.74	2.82*	2.87*
Urea[mmol/l]	7.39	8.37	9.78*	8.79*	7.61

* $P \leq 0.5$ % significance level ** $p \leq 0.01$

F. Urinalysis - not performed.

G. Sacrifice and Pathology:

1. Organ weight -

Compare to controls, absolute liver weights were increased in males (18%) and females (11%) at 14000 ppm. Relative liver weights (vs. body mass) were higher in females at 7000 ppm and above (11% and 13%), and in males at 14000 ppm (17%). The increased relative liver weights of females, which was accompanied by increased plasma cholesterol levels at the same doses, was considered toxicologically significant. Males at 14000 ppm also had significant reductions in absolute (10%) and relative kidney weights. Other organ weights did not differ from the controls.

Table 5. Organ weights

	0 ppm	280 ppm	1400 ppm	7000 ppm	14000 ppm
Males					
Abs. liver wt [mg]	1345	1351	1365	1441	1592*
Rel. liver wt. [mg/100 g bw]	4907	4742	4886	5208	5729**
Abs. kidney wt. [mg]	448	461	443	420	403*
Rel. kidney wt. [mg/100 g bw]	1634	1619	1587	1519	1452**
Females					
Abs. liver weight [mg/100 g bw]	1164	1200	1155	1231	1292
Rel. liver weight [mg]	4976	4929	4913	5509**	5599**

* = $p < 0.5$. ** = $p < 0.01$.

2. Gross pathology -

These were no treatment-related gross necropsy findings.

3. Microscopic pathology -

There were no treatment-related histological changes in all the tissues examined.

III. DISCUSSION

Following oral dietary dosing of mice with SZX 0722 technical for up to 13 weeks, there were no treatment related clinical signs, nor effects on mortality, body weight, or gross or microscopic necropsy findings. The changes in water consumption in males at 7000 ppm and above, and in females at 14000 ppm were considered treatment related, although its toxicological significance remained unclear. The increase in plasma cholesterol in males at 1400 and 7000 ppm but not at 14000, was considered incidental because of the lack of a dose relationship. However, the increase in cholesterol levels of females, at 7000 ppm and above, which was accompanied by increased relative liver weights at the same doses, was considered as indicators of early liver effects. The decreases in absolute and relative kidney weights in males at 14000 ppm were considered on unknown significance, in the absence of any accompanying changes in histology

or pertinent clinical chemistry. There were slight decreases in erythrocyte count and hematocrit and increases in mean corpuscular volume in males at 7000 ppm and above and in females (erythrocyte count and hematocrit only) at 14000 ppm, but the toxicological significance was considered by the study author to be questionable, because the changes were small and without histological correlates

A. Investigators' conclusions.

On the basis of results obtained from this dose-range finding study, dose levels of 0, 280, 1400 and 7000 ppm were considered appropriate for the mouse oncogenicity study.

B. Reviewer comments:

I concur with the study authors conclusion regarding the water intake, organ weight changes and cholesterol levels. However the change in red blood cell parameter decreased erythrocyte count, hematocrit, and MCV are consistent with the expected early changes in anaemia. As such that change is considered toxicologically significant. The increase in cholesterol levels of females, at 7000 ppm and above, which was accompanied by increased relative liver weights at the same doses, was considered toxicologically significant indicators of early liver effects.

The LOAEL for males was 7000 ppm, based on elevated water intake and changes in haematological parameters (erythrocyte count, MCV). The NOAEL in males was 1400 ppm (325.0 mg/kg bw/day),

The LOAEL in females was 7000 ppm, based on increases in liver weights, and plasma cholesterol levels. The NOAEL for females was 1400 ppm (696.5 mg/kg bw/day).

The dose levels for the mouse oncogenicity study which were selected on basis of this dose-range finding study was considered appropriate.

C. Study deficiencies: There were no deficiencies that would affect the acceptability of this dose-range finding study.

IPROVALICARB

Subchronic (13-week) dietary study in mice: **MRID No. 44865711**

Week 13 Body Weights for Mice
(refer to organ weight table DER p. 10)

	Males					Females				
ppm	0	280	1400	7000	14000	0	280	1400	7000	14000
BW g	27.5	28.5	28.0	27.7	27.8	23.4	24.3	23.5	22.3	23.1

10 mice/sex/group
BW g = body weight in grams
Data from DER page 7.